Laboratory Culture of the Aeolid Nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia): Some Aspects of Its Development and Life History

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Abstract. Adult *Berghia verrucicornis* individuals lay white, spiral egg masses containing zygotes. Egg masses are easily cultured in aerated, Millipore-filtered, seasoned aquarium water. Development proceeds quickly, with the bilobed velum apparent by the end of the second day, and the larval shell appearing at the beginning of the third day after oviposition. Hatching occurs 11 to 12 days after oviposition (23.9 ± 1.3°C). If egg masses are incubated without aeration, poecilogonous development is observed; both larvae and juveniles hatch from the same undisturbed egg mass. The larvae metamorphose soon after hatching, losing the velum and larval shell. A habitat-specific inducer is not required for metamorphosis; but a factor associated with the sea anemone *Aiptasia pallida* appears to enhance a larva's tendency to metamorphose. Juveniles begin feeding on *A. pallida* three to four days after metamorphosis. Reproductive maturity is achieved as early as 47 days after oviposition. Because *B. verrucicornis* can be cultured, along with its prey *A. pallida*, at inland facilities, this nudibranch species may be a useful model for laboratory-oriented life history and neurobiological investigations.

Introduction

Successful opisthobranch culture has been limited to species with life histories that tie them to fresh seawater. Among the limiting factors have been: (1) a planktotrophic

larval stage that requires long-term culture best accomplished with fresh, natural seawater, or (2) a prey species for both juveniles and adults that cannot be easily cultured in sufficient quantity in the laboratory (Kriegstein *et al.*, 1974; Harris, 1975; Kempf and Willows, 1977; Switzer-Dunlap and Hadfield, 1977; Bickell and Kempf, 1983; Paige, 1988). Thus, an opisthobranch species that could be reliably cultured through successive generations in a laboratory environment lacking ready access to fresh seawater would be a valuable source of developmental stages, juveniles, and adults for research in such diverse areas as behavior, development, and ecology (*e.g.,* Bonar and Hadfield, 1974; Kandel, 1979; Todd, 1981; Marcus *et al.,* 1988; Kempf, 1989a; Kempf and Todd, 1989). In particular, opisthobranch mollusks have become premier models for neurobiological investigations, because neurons in their central nervous system are large, repeatably identifiable, and easily manipulated (Willows, 1971, 1973; Kriegstein, 1977a, b; Hening *et al.,* 1979; Jacob, 1984; Kempf *et al.,* 1987; Cash and Carew, 1989; Chia and Koss, 1989; Marois and Carew, 1989).

We selected the aeolid nudibranch *Berghia verrucicornis* as a likely candidate for successful laboratory culture. This mollusk occurs on coral rubble in the shallow waters of southern Florida and feeds on the common anemone *Aiptasia pallida.* The adult attains lengths of 2–3 cm, and its dorsum is covered with cerata that appear brown when the nudibranch has been feeding. Among the characteristics that anticipate the successful culture of this species in laboratories lacking a ready supply of fresh sea water are: (1) a short generation time (adulthood is reached five to six weeks after oviposition), (2) lecithotrophic larvae that undergo metamorphosis within days of hatching from the egg mass, and (3) a juve-

nilc and adult prey that can also be reared in the laboratory.

We suggest that *Berghia verrucicornis* could be a useful opisthobranch species for investigators at inland, as well as marine facilities. Herein we describe the culture techniques used to rear and maintain this nudibranch in the laboratory and give an overview of embryonic and larval development. Certain life history traits, such as: (1) induction of metamorphosis; and (2) the potential for developmental variability in this species (poecilogony), are also discussed.

Materials and Methods

Collection of adult animals

Adult individuals of *Berghia verrucicornis* were collected in southern Florida, in abandoned coral quarries on Grassy Key and near Bahia Honda Key, at depths of less than 6 feet during late December 1987, 1988, and March 1988, 1989. They were transported to Auburn, Alabama in aerated buckets of seawater with fresh seawater changes every 4–5 h. The sea anemone *Aiptasia pallida* was also collected in the Florida Keys and transported to Auburn University as food for the juvenile and adult *B. verrucicornis*.

Culture of the sea anemone Aiptasia pallida

Aiptasia pallida may be cultivated in typical salt-water aquaria with undergravel filtration; wet/dry trickle filters should also be adequate. Our own system consists of a number of individual aquaria, as well as a large-scale culture system consisting of one 110-gallon, four 30-gallon, and two 20-gallon aquaria connected together with flow-through water circulation. One week after set-up, new aquaria with undergravel filtration are conditioned by the addition to each of a few small salt-water fish (*e.g.*, clown-fish or damsel fish) or invertebrates (*e.g.*, hermit crabs, anemones). These animals are fed and maintained for at least one month; their metabolic and digestive wastes provide for the growth of essential, gravel associated, bacterial populations that detoxify ammonia and nitrites.

At the end of the conditioning period, a number of *Aiptasia pallida* (20–30) are added to each aquarium. Anemones may be obtained from the field or from various suppliers such as Carolina Biological Supply Co. The anemones are maintained under a combination of "Grow-Lux" and Actinic Blue fluorescent lighting and are fed newly hatched brine shrimp every two days. With appropriate care, the number of *A. pallida* will gradually increase as clones develop from pedal laceration. Regular replenishment of aquarium water with freshly prepared, artificial seawater is important. Individual aquaria are most easily replenished each day, when "seasoned aquarium water"

is removed for use in the culture of egg masses, larvae, juveniles, and adult *Berghia verrucicornis* (Fig. 1A, B, C). Healthy anemone colonies should be established before an attempt is made to establish a colony of *B. verrucicornis*. Other, more detailed, methods for culture of *Aiptasia* have been described by Hessinger and Hessinger (1981).

Culture and feeding methods for Berghia verrucicornis

Figure 1 summarizes the culture methods used. Pairs of adult *Berghia verrucicornis* used for egg mass production were kept in glass bowls containing 300–350 ml of 0.45 μm Millipore-filtered artificial seawater (Instant Ocean or Tropic Marine Systems, Inc.) that was obtained from established saltwater aquaria supplied with $CaCO_3$ gravel and undergravel filtration. This water is designated as "Millipore-filtered, seasoned, aquarium water" (MFSA). The water and bowl were changed daily for each culture. As opisthobranch egg masses were layed, they were transferred either to aerated 500-ml beakers containing 350 ml of MFSA or, in the case of experiments concerned with direct development, to unaerated 300-ml glass crystallizing dishes containing 100 ml of MFSA. Water and containers were changed daily. Harvested *Aiptasia pallida*, used for food, were also kept in bowls of MFSA; both bowls and water were changed every few days.

Two days before the expected date of hatching, two or three small *Aiptasia pallida* were placed in fresh dishes containing MFSA. The MFSA in these cultures was changed each day. When the larvae hatched, we concentrated them by pouring the culture water through a Nitex strainer (see Switzer-Dunlap and Hadfield, 1981); we then pipetted them into the dishes containing anemones, taking care to release them underwater so that they would not be trapped at the air-water interface. The number of larvae in each metamorphosis dish varied depending upon the experiment.

Metamorphosis usually occurred in one to three days, and crawling juveniles were present by the third day. After a successful metamorphosis, and until the nudibranchs began feeding, the culture water was changed on alternate days. Thereafter, sea anemones were added as needed, and the culture water and container were changed daily. See Figure 1 for additional details about the frequency with which the water and culture containers may be changed for early juveniles.

Antibiotics were not used in any stage of culture. Regular water changes were sufficient to prevent problems with protist and bacterial contaminants.

Embryonic and larval development

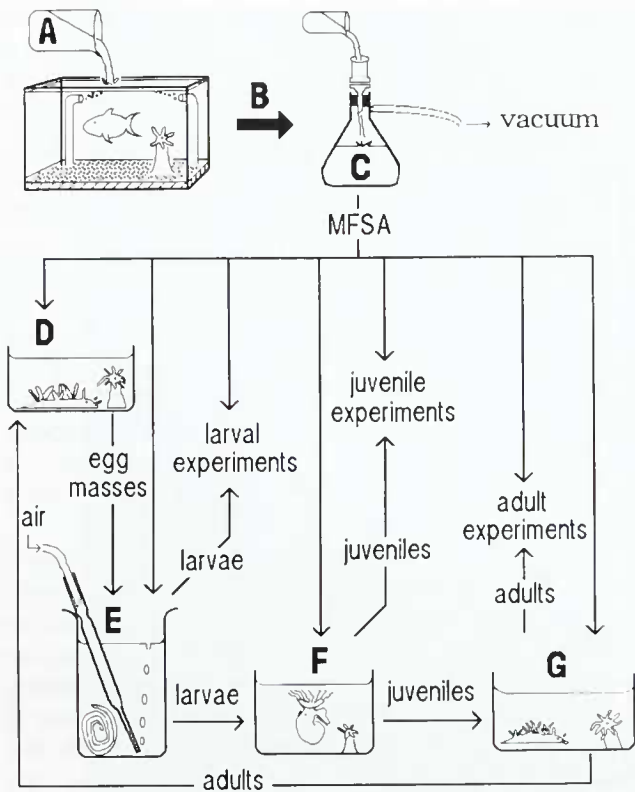Zygotes could only be obtained from egg masses that had been collected directly after oviposition. At this time,

Figure 1. Algorithm for the culture of *Berghia verrucicornis*. (A) Aquaria with undergravel filtration and a 3-4 inch thick bed of CaCO$_3$ gravel are filled with freshly prepared artificial seawater. A few invertebrates or fish are added to each aquarium after 3-7 days of operation. After one month of operation, additional anemones or other animals are added to the aquaria. Salinity is adjusted each week. Water from these aquaria is designated as "seasoned aquarium water." (B) Seasoned aquarium water is regularly removed from established aquaria and used for egg mass, larval, juvenile and adult cultures. (C) Seasoned aquarium water is filtered through a 0.45-μm Millipore filter for culture use. (MFSA: Millipore-filtered seasoned aquarium water.) (D) Adults for stock cultures are maintained in small bowls (about 13 cm diameter, 5 cm deep). Bowl and water are changed daily. Appropriately sized anemone prey are added as needed. (E) Egg masses are collected from adult cultures and placed in 500-ml beakers containing ~350 ml of MFSA. Aeration is provided through Pasteur pipettes. Beaker and water are changed daily. As egg masses hatch, larvae are concentrated with a Nitex filter (see Switzer-Dunlap and Hadfield, 1981, p. 208) and used in larval experiments or metamorphosis cultures. (F) For metamorphosis cultures, larvae are placed in crystallizing dishes containing MFSA and a few small anemones. Water is left unchanged for 5-10 days and then the water is changed daily, and the bowl every few days as needed, until juveniles are large enough to transfer to finger bowls (2-3 weeks after metamorphosis). Juveniles are harvested for experiments or stock juvenile cultures. (G) Juvenile stocks are cultured as are the adults (see D above). Appropriate sized juveniles or adults are used for experiments or stock adult cultures. Transfer of larger juveniles and adults between cultures is easily accomplished with a "reversed" Pasteur pipette, the rubber bulb being placed over the end from which the tapered tip has been broken off.

the egg could be observed with a dissecting microscope, and its diameter measured with an ocular micrometer. Ten egg masses were cultured as described above and were observed at hourly intervals with a compound microscope until the cleavage divisions were finally obscured by the growing number of cells. Subsequently, until hatching, observations were made several times a day so that the appearance of characteristic larval structures, such as the velum, foot, left and right digestive diverticula, larval retractor muscles, eyespots, and propodium could be recorded. The number of egg masses laid per week was recorded for more than a month, for 22 adult nudibranchs.

Induction of metamorphosis

We assessed the effect of the sea anemone *Aiptasia pallida* on metamorphosis, by observing the following four metamorphosis cultures each containing 50 larvae: (1) MFSA in dishes containing the anemone *A. pallida* (MFSA + A); (2) MFSA in dishes without *A. pallida* (MFSA − A); (3) a bacterial film culture (BF) (see below); and (4) MFSA from an aquarium that had never contained *A. pallida* (*Aiptasia*-free, seasoned, aquarium water, AFSA). MFSA for treatments 1, 2, and 3 was taken from aquaria that contained *A. pallida*. Twenty replicates were performed for treatments 1 and 2, and ten replicates for treatments 3 and 4. New glassware was used to filter water and hold AFSA cultures. The bacterial film culture was prepared by removing a few pieces of gravel from an aquarium containing *A. pallida* and placing them in MFSA overnight. The next day the gravel was removed, the MFSA in the dish was changed and, finally, the larvae added. The number of larvae undergoing metamorphosis in each of the cultures was counted daily over three days. Care was taken to count all juveniles on the bottom, sides, and water surfaces of the culture. The mean ± standard deviation was calculated for each treatment and day. A Student *t*-test was used to determine whether the difference between the means was significant (Steel and Torrie, 1980).

Culture of egg masses for direct development

Single intact egg masses were cultured in dishes of MFSA without aeration. MFSA was changed daily in these cultures; the medium was decanted and fresh MFSA was gently added.

Results

Collection of adult animals

Berghia verrucicornis never inhabits the mangrove roots where its juvenile and adult food, the sea anemone *Aiptasia pallida*, occurs in abundance. Rather, the nudibranch is found in another habitat of *A. pallida*, among coral rubble in shallow, sub-tidal waters. The nudibranchs are relatively difficult to spot on the darkly colored coral rocks because the dorsally positioned cerata appear brown when the animal has been feeding. A typical adult of *B.*

verrucicornis is shown in Figure 2A. The white appearance of its egg mass (Fig. 2B) facilitates the collection of *B. verrucicornis* by providing evidence of the adults' presence.

The egg mass

The gelatinous egg masses are laid as untwisted strings in a dextral or sinistral spiral (Fig. 2B). In the laboratory, each pair of adults (n = 11 pairs) laid an average of 4 ± 1 egg masses weekly. The egg masses were found attached to the sides and bottom of the culture dish, and some were floating at the air-water interface; the site of oviposition seemed unaffected by the presence or absence of *Aiptasia pallida*. In the field, these egg masses are deposited on the underside of coral "rocks."

The embryos, whether in the field or in culture, are contained within two membranes: one, a primary membrane or capsule, surrounds each individual embryo; the secondary membrane encases all of the capsules (Fig. 2B, C). Empty primary egg capsules are located at both ends of the egg string.

Embryogenesis

Egg masses were cultured at 23.9 ± 1.3°C; the range was 21–26°C (Table 1). Cleavage proceeded quickly at this temperature, and because the divisions within a given egg mass were asynchronous, both two-celled embryos and zygotes could be seen. This asynchrony was evident throughout cleavage, until the later blastula stage, when the opacity of the blastomeres reduced the accuracy of the cell count. All of the larvae hatched from the egg mass at the same morphological stage of development.

At 2.2 ± 0.3 days after oviposition, the velar rudiment was evident at the future anterior end of the embryo, and the embryo began to move. At first, the cilia were difficult to detect, and we could not be sure that they were beating. Nevertheless, the cilia are probably the cause of the movement of the embryos.

Larval structures

The velum is the first larval structure evident. It develops as a ridge on the anterior end of the embryo and assumes its characteristic bilobed appearance on the third day. The velar lobes are located anterolateral to the mouth and possess pre-oral and post-oral ciliary bands. By 4.7 ± 0.9 days, the embryo can partially retract the velum into the shell, indicating the presence of a retractor muscle. Large refractile cells, located around the periphery of the velum, are visible from day 8 until metamorphosis. The velum is lost during metamorphosis.

The larval foot (metapodium) appears soon after the velum, during the second day of development (2.8 ± 0.2 days), as a flat, blade-like metapodium. As development

continues, the foot thickens and lengthens. The operculum is present by the fourth day. Cilia appear along the ventral length of the foot on the fifth day. The metapodium thickens considerably by the seventh day, and the posterior aspect develops into a definitive propodium soon thereafter (7.4 ± 0.5 days). During metamorphosis the foot becomes longer and wider; eventually it occupies the ventral surface of the juvenile.

The larval shell appears concurrently with the foot. The shell is clear, allowing an unobstructed view of the larval viscera. Shell length increases from 143.7 ± 21.4 μm on day 3 to a plateau of 251.4 ± 7.0 μm on day 8 (Fig. 3). When shell deposition has been completed, the mantle fold begins to withdraw from the shell edge. This occurs 7.0 ± 0.3 days after oviposition. The shell stopped growing once the mantle fold retracted, and was shed by the larva during metamorphosis.

The larval gut of *Berghia verrucicornis* occupies most of the space within the shell in the early days of development. It appears milky white and presumably contains yolk reserves that maintain the nudibranch throughout its embryonic and larval periods, and probably during early juvenile development. The various components of the viscera were not discernible early in development, but became evident 4.1 ± 0.2 days after oviposition. The stomach and the left and right digestive diverticula constituted most of the viscera. Initially, those three visceral organs are of similar size. As development proceeds, the right digestive diverticulum decreases in size (from 93.7 ± 9.6 μm on day 4, to 31.6 ± 5.0 μm on day 10), presumably reflecting the use of yolk reserves in this structure. The size of the left digestive diverticulum remains unchanged throughout development; Figure 4 shows the relationship between the diverticula from day 4 to day 10. The intestine could be seen looping anteriorly, from the posterior portion of the stomach to the anus on the right side of the mantle cavity, at the same time that the stomach and digestive diverticula were evident.

Sense organs present in the embryo and larva are the eyespots and statocysts. The statocysts are situated in the larval foot near its attachment to the body; they become apparent four days after oviposition. The two eyespots are located dorsally, directly posterior to the velar lobes; they appeared 6.3 ± 0.4 days after oviposition.

Hatching

Hatching occurred 11.6 ± 0.5 days after oviposition; the range was 9–14 days (T = 23.9 ± 1.3°C). In aerated laboratory cultures, the secondary egg mass membrane begins to break down first, releasing intact primary egg capsules. These primary egg capsules appear more pliable than earlier in development and change shape as the embryo moved within them. The primary egg capsules soon
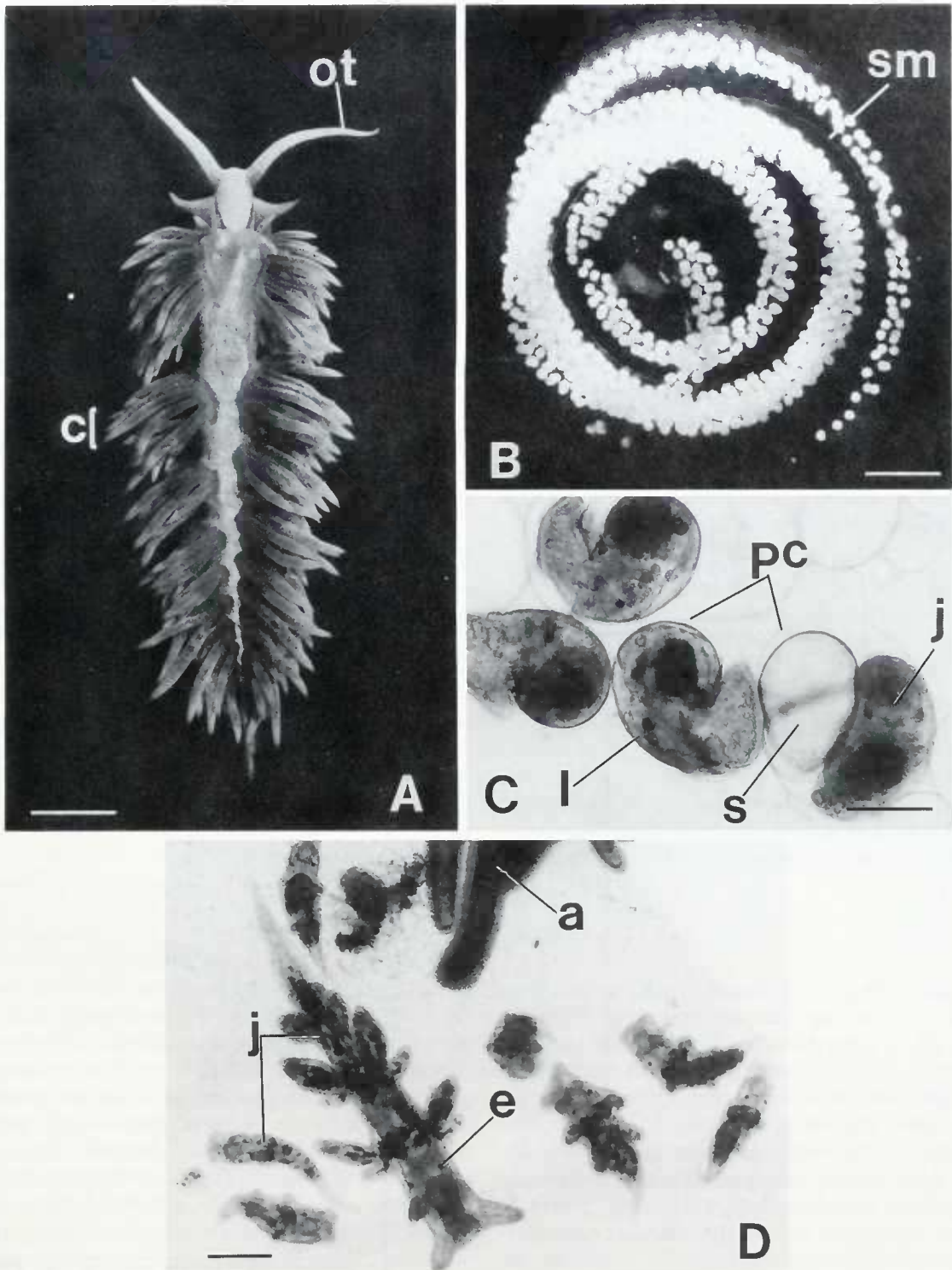
Figure 2. Photographs of several stages in the life history of *Berghia verrucicornis.* (A) Adult specimen. Scale bar = 2.0 mm. (B) Egg mass. Scale bar = 2 mm. (C) Example of direct development. A newly metamorphosed juvenile is still present in an egg capsule. Scale bar = 140 μm. (D) Juveniles of *Berghia verrucicornis.* Scale bar = 0.3 mm. Legend: a = anemone; c = ceratal tuft; e = eyespot; j = juvenile; l = larva; ot = oral tentacle; pc = primary capsule membrane; sm = secondary membrane; s = larval shell.

Time* (h. days)		Developmental event
$T = 23.9 \pm 1.3°C$		
0 h	(n** = 10)	Oviposition
3.4 ± 0.8 h	(n = 10)	First cleavage
14.5 ± 4.1 h	(n = 10)	Blastula
1.3 ± 0.2 days	(n = 10)	Gastrula
2.2 ± 0.3 days	(n = 10)	Velar rudiment appears
2.8 ± 0.2 days	(n = 10)	Shell and foot visible
3.3 ± 0.2 days	(n = 9)	Velum assumes bilobed shape
4.1 ± 0.2 days	(n = 9)	Viscera differentiated into stomach, and left and right digestive diverticula
4.7 ± 0.9 days	(n = 7)	Larva able to partially retract velum into shell, indicating presence of larval retractor muscle
5.5 days	(n = 2)	Cilia apparent on posterior aspect of metapodium
6.3 ± 0.4 days	(n = 9)	Eyespots visible
7.0 ± 0.3 days	(n = 9)	Mantle retracts from shell aperture
7.4 ± 0.5 days	(n = 9)	Propodium develops on anteroventral aspect of metapodium
11.6 ± 0.5 days	(n = 8)	Hatching
13.0 ± 0.9 days	(n = 8)	Metamorphosis
15.3 ± 0.8 days	(n = 6)	Juvenile begins feeding

* Times are given as mean ± standard deviation.

** n refers to the number of egg mass cultures observed; four cultures were discontinued during the observation period.

rupture, releasing veliger larvae. Neither the mouth nor the foot appear to participate actively in the hatching process.

Metamorphosis

The lecithotrophic larval stage of *Berghia verrucicornis* is released from the egg mass and undergoes metamorphosis as early as 1 day thereafter. Within metamorphosis cultures, the larvae swim vertically upward immediately after release from the primary egg capsules, and occasionally become trapped at the air-water interface. The larvae trapped at the water surface can move around because the velar cilia are submerged. They are apparently able to undergo metamorphosis without the benefit of attachment of the foot to the substratum. In metamorphosis cultures containing the anemone *A. pallida*, the larvae show no preference to settle near the anemones.

Marked changes in morphology take place during metamorphosis. After a short planktonic larval phase, generally 1–3 days, the larvae settle on the bottom and sides of the metamorphosis dish. Once settled, they appear to crawl randomly along the bottom, slowly beating their velar cilia. The velum is lost first, and the larvae continue to crawl on the substratum with the shell still attached. Eventually, the shell is cast off, and over the next few hours the body lengthens as the final vermiform morphology is assumed.

Various culture treatments were tested as inducers of metamorphosis (Fig. 5). The number of larvae completing metamorphosis was always greatest in the presence of the anemone *Aiptasia pallida* (MFSA + A culture). The mean cumulative number of larvae metamorphosing in MFSA + A cultures was significantly greater than the number of larvae undergoing metamorphosis in MFSA − A cultures ($P < 0.001$ for Day 1; $P < 0.01$ for Day 2 and 3) or in the AFSA cultures ($P < 0.01$ for all three days). However, cultures containing anemones (MFSA + A) did not differ significantly from the bacterial film (BF) cultures over the three day observation period ($P > 0.05$).

A comparison of the number of metamorphoses in the control cultures (BF, AFSA, and MFSA − A), revealed that the BF and AFSA cultures were significantly different only on Day 1 after hatching ($P < 0.05$). No difference was seen between the BF cultures and the MFSA − A cultures on any day tested ($P > 0.05$). Similarly, the number of larvae metamorphosing in the AFSA cultures was, statistically, the same as in cultures containing MFSA − A ($P > 0.05$).

Direct development

The hatching times for veliger larvae in aerated and unaerated cultures were not different ($P > 0.5$). The av-
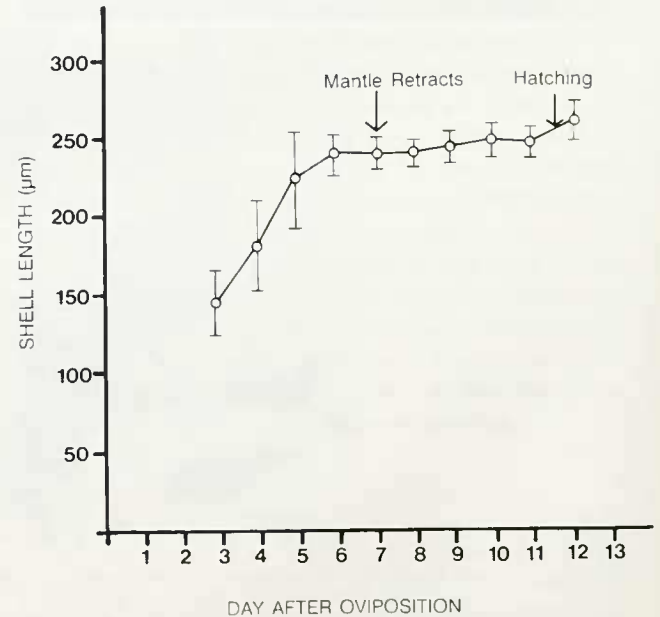

Figure 3. Changes in shell length during embryonic and larval development. n = the number of shell lengths measured.

Day =	3	4	5	6	7	8	9	10	11	12
n =	26	80	95	100	91	96	81	50	30	10

conditions were identical for each egg mass. The external appearance of juveniles was identical, whether development was direct or via a lecithotrophic larva.

Juvenile and adult

Newly metamorphosed juveniles were oval and white, with eyespots indicating the anterior end, and the remnants of the site of larval attachment to the shell at the posterior end. The juveniles initially crawled randomly over the bottom of the culture dish without feeding. One day after metamorphosis, a slender tail-like extension of the foot projected posterior to the elongated body of the juvenile. Rhinophore rudiments were present as small dorsal projections anterior to the eyes at this time. Stiff, cirrus-like projections extended from the anterior end of the juvenile and in opposed pairs along the dorsum. The foot was tightly associated with the body along the entire ventral length of the juvenile. In culture dishes, the juveniles gathered at the base of the anemones one day after metamorphosis. They then dispersed before returning one to two days later to begin feeding (15.3 ± 0.8 days after

Figure 4. Changes in the diameter of the left and right digestive diverticula during embryogenesis. LDD, left digestive diverticulum; RDD, right digestive diverticulum. n = the number of diverticula measured.

Day =	4	5	6	7	8	9	10
n_{LDD} =	10	40	60	61	60	60	40
n_{RDD} =	10	20	30	31	30	18	5

erage time from oviposition to hatching in unaerated cultures was 11.7 ± 1.1 days (n = 30); in aerated cultures it was 11.6 ± 0.5 days (n = 8). However, both lecithotrophic larval and direct development were seen in egg masses from unaerated cultures (Fig. 2C). This type of variable development is known as poecilogony, which has been defined as "intraspecific variation in the mode of larval development" (Bouchet, 1989). In some instances, metamorphosis occurred within the primary egg capsule and the individuals hatched as juveniles, leaving the shell in the egg capsule; other capsules released lecithotrophic larvae that could metamorphose soon thereafter. Intracapsular metamorphosis occurred after some of the larvae had already hatched from the same egg mass. Veliger larvae hatching from the same egg masses underwent metamorphosis after a short planktonic period. The total number of hatchlings of each type varied considerably from one egg mass to the next. This may be due to differences between the embryos themselves, as the culture
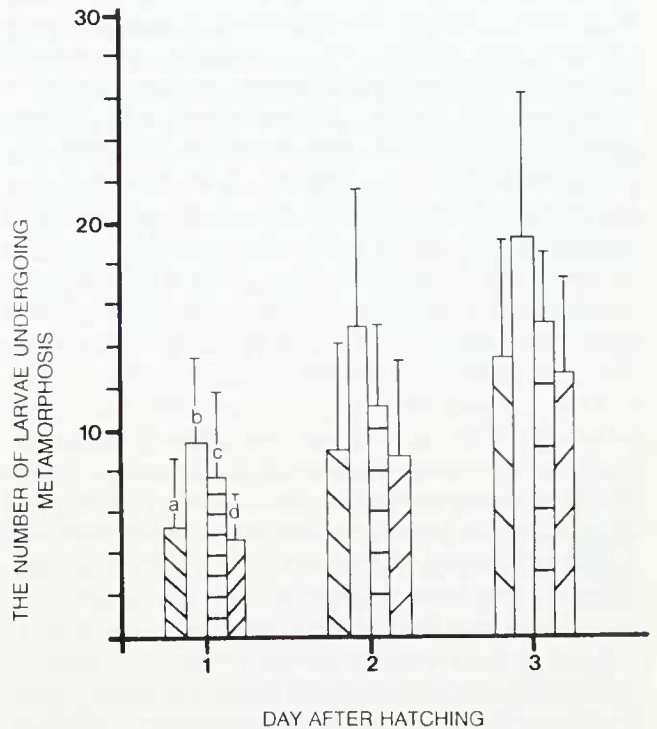
Figure 5. The mean cumulative number of larvae undergoing metamorphosis in four different culture media. Each culture contained 50 larvae initially. a = MFSA from aquaria containing *Aiptasia pallida*, but no anemones present (MFSA − A); b = MFSA plus the sea anemone *A. pallida* (MFSA + A); c = bacterial film culture (BF); d = *Aiptasia*-free, seasoned, aquarium water (AFSA). Twenty replicates were performed for treatments a and b, and 10 for treatments c and d. Error bars represent one S.D.

oviposition). The paired, dorsal cerata appeared 8–11 days after metamorphosis (Fig. 2D). Reproductive maturity was reached 50 ± 3 days after oviposition, but had been observed in the laboratory as early as 47 days. The first egg masses were small and contained less than 100 embryos.

Discussion

Berghia verrucicornis is the first opisthobranch mollusk to be cultured through successive generations and maintained as a viable population of experimental animals at an inland facility. This species has several characteristics that suggest it will be a useful model for laboratory oriented research. These include ease of maintenance, regular oviposition throughout the year ($3 +$ egg masses/pair of animals/week), a prey organism (*Aiptasia pallida*) that can be cultured in the lab, a short embryonic (9–14 days) and lecithotrophic larval period (1–3 days), and a generation time (egg to egg) as short as 47 days. These traits should make *B. verrucicornis* a convenient organism for research in larval ecology, energetics, neurodevelopment, and, probably, neurophysiology. In addition, this species appears to maintain a symbiosis with a zooxanthella that it obtains from its prey (Kempf, 1989b), so the association can be used to investigate the establishment, energetics, and evolution of algal-invertebrate endosymbioses.

In general, the development of *Berghia verrucicornis* follows that reported for other opisthobranch mollusks with lecithotrophic larvae (Thompson, 1958, 1962; Bonar and Hadfield, 1974; Harris, 1975; and others). It is characterized by the major life cycle stages that Kriegstein (1977b) noted for *Aplysia californica, i.e.,* (1) embryonic, (2) planktonic, (3) metamorphic, (4) juvenile, and (5) adult. The morphological descriptions given for posthatching stages of *A. californica* cannot be applied directly to *B. verrucicornis* because the larvae of *B. verrucicornis* are lecithotrophic and undergo what might be considered homologous developmental stages as an embryo, rather than as a feeding larva. In lecithotrophic larvae, such as these, the major increase in size of the viscera, development of the eyespots and propodium, and maximum shell length are all attained prior to hatching. In opisthobranch species with obligate planktotrophic larvae, such as *A. californica,* these events occur after hatching. Thus, *B. verrucicornis* might be included in the non-feeding, non-growing group of veliger larvae proposed by Hadfield (1963). But more recent investigations into the feeding potential of lecithotrophic larvae suggest that such distinctions may be hazy at best, because the lecithotrophic larvae of at least some species are capable of feeding if the opportunity presents itself (Kempf and Hadfield, 1985; Emlet, 1986; Kempf and Todd, 1989). A safer developmental designation would be that of type 2 larval devel-

opment proposed by Thompson (1967); these larvae hatch from their egg capsules as late veligers, and undergo metamorphosis shortly thereafter.

The early cleavages of the aeolid nudibranch *Phestilla melanobranchia* are synchronized (Harris, 1975). Conversely, early cleavages in egg masses of *B. verrucicornis* are asynchronous. For instance, in a given egg mass, both zygotes and two-celled embryos can be observed simultaneously, although embryos more than one cleavage stage apart are never observed. Since this asynchrony in developmental stage is seen in embryos situated side by side, it is probably not due to differences in oxygen diffusion through the egg mass (Chaffee and Strathmann, 1984; Strathmann and Chaffee, 1984). Mediation of early development by a cue intrinsic to the egg mass (Harris, 1975) is a possibility, though the early development is certainly not as well synchronized as that reported for *P. melanobranchia.* As development proceeds, the discrepancy in cleavage rates becomes less noticeable, with later stages of *B. verrucicornis* developing at the same apparent rate. As a result, all sibling embryos incubated in an aerated culture hatch together at essentially the same morphological stage of development.

Lecithotrophic development, such as that characterized by the embryos and larvae of *Berghia verrucicornis,* is generally thought to use maternally derived yolk reserves to fulfill energetic needs. Recent investigations (Jaeckle and Manahan, 1989; Manahan, 1989; Manahan *et al.,* 1989; Shilling and Manahan, 1990) indicate that cellular endocytic systems in tissues other than those of the digestive tract may allow dissolved organic material from surrounding seawater to make a significant contribution to the energetic requirements of larval and possibly embryonic development. Although no conclusions with respect to the developmental importance of DOM can be drawn from this study, the decrease in size of the right digestive diverticulum during embryonic development in *B. verrucicornis* suggests that yolk reserves stored in this organ are an important nutrient source used to support embryonic energetic needs.

The mechanism of hatching has been described for several opisthobranchs. The aeolid, *Phestilla melanobranchia,* hatches through a hole made in the capsule wall by repeated contact with the mouth (Harris, 1975). In *Adalaria proxima,* a dorid, hatching appears to result from mechanical buffeting of the primary capsule wall with the velar cilia and, perhaps, the secretion of an enzyme, because the capsule wall becomes more pliable as the hatching date approaches (Thompson, 1958). Larvae of *Berghia verrucicornis* appear to effect their release from the primary egg capsule in a manner similar to that described for *A. proxima.* As hatching approaches the capsule membrane becomes more flexible. Continuous buffeting and distortion of this barrier by the velar cilia may be

sufficient to eventually tear an opening that allows release of the larva. Bacteria, protozoans, and other microfauna may also contribute to the lysis of egg mass membranes during hatching.

In opisthobranchs, as in many other invertebrates, external cues are often responsible for the onset of metamorphosis (Thorson, 1946; Thompson, 1958, 1962; Bonar and Hadfield, 1974; Harris, 1975). Metamorphosis of the larvae of such species is triggered by chemicals or factors associated with a specific aspect of the mature animals habitat, often the presence of the food of adults (Hadfield, 1977, 1984, 1986; Burke, 1983, 1986; Hadfield and Scheuer, 1985; Morse, 1985; Fitt *et al.*, 1987; and many others). Our experiments demonstrate that a habitat-specific inducing factor is not an absolute requirement for *Berghia verrucicornis*. A mean of 26% of the veligers released from the egg masses of this species metamorphosed in the absence of a habitat-specific inducing molecule (AFSA cultures). But the treatments used to examine induction do not preclude the presence of some ubiquitous inducing molecule common to seawater or to habitats in general (*e.g.*, from bacterial or algal films). Further analysis of our experiments on metamorphic induction requires consideration of three components of the results. (1) The presence of the anemone *Aiptasia pallida* always resulted in the greatest number of larvae completing metamorphosis. (2) The number of larvae metamorphosing in anemone-containing treatments was always significantly different from that in controls containing only (a) Millipore-filtered, seasoned aquarium water from aquaria containing *A. pallida* (MFSA – A), or (b) *Aiptasia*-free, seasoned aquarium water (AFSA). (3) Bacterial film controls were only different from the AFSA controls on the first day after hatching. These results suggest that a factor (presumably chemical) enhancing a larva's tendency to metamorphose is associated with the anemone *A. pallida*. The enhancement of metamorphosis by a substance associated with the prey of juveniles and adults, rather than a habitat-specific metamorphic induction, would be consistent with proposals recently made by Kempf and Todd (1989) concerning the functional aspects of evolutionary selection for direct development (see below).

The juveniles that gather at the base of the anemones one day after metamorphosis may be responding to the same anemone-associated factor that enhances metamorphosis. The presence of such a response would help to ensure that they gathered in areas containing their prey organism. After the initial "discovery" of the anemone, juveniles in culture bowls disperse, presumably searching out other areas where prey are present. The re-aggregation of juveniles at the bases of culture bowl anemones 1–2 days after dispersal may well be an artifact produced by confinement in the culture container. The nudibranchs simply "rediscovered" the same anemones in the small

container and began feeding as their maternally derived yolk reserves were depleted.

Classically, most invertebrate species have been considered to undergo a single type of development; but reports of poecilogony occurring in a number of invertebrate species, including opisthobranchs, suggest that intraspecific developmental flexibility is greater than was previously thought possible (Clark *et al.*, 1978; Eyster, 1979; Gibson and Chia, 1989; Hoagland and Robertson, 1988 review). Our observations indicate that, when egg masses of *Berghia verrucicornis* are agitated by aeration, only veliger larvae hatch from the primary egg capsules. An inadvertently forgotten culture in our lab led to the serendipitous observation that, if egg masses of this species are left undisturbed by aeration, both veligers and juveniles will be released from primary capsules of the same egg mass. Repeated observations of additional unaerated egg masses suggest that the factors affecting the type of development expressed by egg masses of *Berghia verrucicornis* may be extrinsic rather than intrinsic. One explanation for this phenomenon is that aeration of egg mass cultures causes a mechanical break down of egg mass membranes, resulting in the "premature" release of only veliger larvae from the primary egg capsules. Lack of aeration (*i.e.*, agitation) would allow for greater integrity of the egg mass membranes, thus allowing time for some embryos to undergo metamorphosis within the primary capsule. This scenario seems unlikely, because aerated and unaerated cultures show no significant difference in time to hatching at similar temperatures. Perhaps a more acceptable explanation is that agitation due to aeration somehow interferes with either (a) the physical act of metamorphosis itself or, (b) the intrinsic larval systems responsible for attaining competence or initiating metamorphosis. Whether the poecilogony described above actually occurs in the field has not been determined; but, two facts suggest that it does: (1) *Berghia verrucicornis* lays its egg masses closely attached, along the egg string's entire length, to the bottoms of coral rocks, thus reducing the effects of agitation; and (2) egg masses are laid in subtidal habitats of low energy flux in some instances. Thus, *Berghia verrucicornis* may enjoy the best of two worlds, being able to benefit from both the advantages of direct development and dispersal via a larval stage.

Kempf and Todd (1989) have proposed that, as a species evolves toward a reproductive strategy characterized by lecithotrophic development, a loss in habitat-specific induction of metamorphosis would usually be necessary prior to the establishment of a direct mode of development. *Berghia verrucicornis* appears to exhibit developmental characteristics that are consistent with this hypothesis. It has lost the need for a habitat-specific inducer and, given the right environmental conditions, can reproduce via both a larval stage and direct metamorphic

development as described by Bonar (1976). As such, *B. verrucicornis* appears to be poised on the cusp between indirect (larval) and direct development, suggesting that it will be useful in future studies concerning the evolution of invertebrate reproductive strategies.

Acknowledgments

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